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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/017,718	12/14/2001	Karl H. Weisgraber	UCAL-222	5282

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EXAMINER	
TON, THAIAN N	

ART UNIT	PAPER NUMBER
1632	9

DATE MAILED: 10/23/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/017,718	WEISGRABER ET AL.	
	Examiner	Art Unit	
	Thái-An N. Ton	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *18 June 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-19* is/are pending in the application.

4a) Of the above claim(s) *5-13 and 16-19* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-4, 14, 15* is/are rejected.

7)☒ Claim(s) *15* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *12/14/01* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *5,6* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Claims 1-19 are pending. Claims 1-4, 14 and 15 are under current examination.

Election/Restrictions

Applicant's election with traverse of Group I (claims 1-4, 14 and 15) in Paper No. 8 is acknowledged. The traversal is on the ground(s) that that it would not be unduly burdensome to perform a search on all of the pending claims, and that Applicants request the rejoinder of at least Groups I and II because they are of the same class and subclass. This is not found persuasive because the Groups I and II are directed to materially different and separate protocol, which require different technical considerations. Furthermore, the inventions recited in the Restriction requirement have acquired a separate status in the art as a separate subject for inventive effort and require independent searches. The search for each of the inventions is not co-extensive particularly with regard to the literature search. Further, a reference which would anticipate the invention of one group would not necessarily anticipate or even make obvious another group.

The requirement is still deemed proper and is therefore made FINAL.

Claims 5-13 and 16-19 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected groups, there being no allowable

generic or linking claim. Applicant timely traversed the restriction (election) requirement in Paper No. 8.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Information Disclosure Statement

Applicants' Information Disclosure Statements, filed 3/5/02 and 10/15/02, have been considered.

Claim Objections

Claim 15 is objected to because of the following informalities: the claim is dependent upon itself. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-4, 14 and 15 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a transgenic mouse whose

genome comprises an endogenous mouse apolipoprotein E4 [apoE4] allele operably linked to endogenous regulatory sequences, wherein the endogenous apoE4 allele has been modified by the substitution of an arginine residue with a threonine reside at the position equivalent to amino acid 61 of human apoE4 and wherein the expression of the modified endogenous mouse apoE4 results in the preferential binding of lower density lipoproteins, when compared to a wild-type mouse, does not reasonably provide enablement for gene-targeted non-human animals comprising a modified endogenous apoE allele, wherein the modified allele comprises an apoE-encoding nucleic acid under transcriptional control of endogenous regulatory sequences, and wherein the modified allele encodes a modified apoE that exhibits domain interaction characteristic of human apoE4. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The claims are directed to a gene-targeted non-human animal comprising a modified endogenous apoE allele, wherein the modified allele comprises an apoE-encoding nucleic acid under transcriptional control of endogenous regulatory sequences, and wherein the modified allele encodes the modified apoE that exhibits domain interact characteristic of human apoliprotein E4.

The specification teaches the generation of a non-human gene-targeted animal for the study of apolipoprotein E4 [apoE4] pathologies, wherein the

endogenous apoE of the gene targeted animal is genetically altered such that the encoded recombinant apoE polypeptide exhibits domain interaction. It is this domain interaction that is representative of human apoE4 domain, and as such, these animals can be used as models for human apoE4 domain interaction. In particular, the specification teaches a gene-targeted mouse comprising a modified mouse apoE4 gene, wherein the modification comprises a Thr → Arg substitution at a position equivalent to the amino acid 61 of human apoE4. See p. 6, ¶ 0020. The specification teaches that the production of the gene-targeted non-human animals by utilizing ES cells [see pp. 20-21]. In particular, the specification teaches the generation of the mouse ApoE4 gene-targeting vector. The mouse ApoE gene contains four exons and three introns, and the codon for the Thr-61 human-equivalent is located at the end of the third exon, exactly as it is for the human gene. Mutation of the Thr→Arg was introduced and a thymidine kinase [TK] marker used as a negative selection marker and the *Neo* gene used as a positive selection marker. The *Neo* gene was flanked with loxP sites. The targeting vector was electroporated into mouse ES cells, which were then selected against TK incorporation, and then for neomycin resistance. Heterozygous cells were then injected into mouse blastocysts and then the blastocysts implanted into pseudopregnant female mice, wherein three chimeric male mice were obtained which displayed germline transmission of the targeted allele. See pp. 53-54. The levels of mRNA of apoE were compared between wild-type and homozygous Arg-61

mutant mice and it was found that expression was restored in all apoE-expressing tissues and organs and that the mRNA expression levels in the Arg-61 mice were identical to the levels in wild-type mice. See p. 54, ¶ 190.

The domain interaction in the mutant mouse Arg-61 was determined using *in vitro* lipoprotein binding assays and *in vivo* by the effects of Arg-61 mutant apoE on plasma lipoprotein metabolism. In humans, the plasma levels of apoE4 are lower than that of apoE3 and apoE2. Isoelectric focusing was performed on +/+, +/- and -/- apoE4 Arg-61 mutant mice. It was found that that the Arg-61 mutation resulted in the positive direction, which confirms the expression of the mutant mouse apoE gene produces mutant Arg-61 protein. Additionally, it was found that the amount of Arg-61 apoE in heterozygous mice was reduced by approximately 70% in the plasma, when compared to wild-type mice, whereas the mice had equal levels of Arg-61 and wild-type apoE in their cerebrospinal fluid, which does not contain VLDL or any apoB-containing lipoproteins. Primary hepatocytes were cultured and the relative amounts of the two mouse isoforms secreted in the medium were determined by isoelectric focusing. It was found that equal amounts of each isoform were secreted by the hepatocytes. See pp. 54-55. The distribution of lipoprotein fractions from +/- mouse plasma was examined by separation by gel filtration. Mice transport more than 85% of their cholesterol in HDL, and as such, have a much lower plasma concentration of apoB-containing VLDL, IDL and LDL than humans. The +/- mice were fed an atherogenic diet for 6 days to increase the

plasma concentration of apoB-containing lipoproteins. It was found that wild-type mice distribute apoE differently than +/- mice [See Figure 3B], with the majority of the Arg-61 in the lower density apoB-containing lipoproteins. The binding of recombinant Arg-61 mouse apoE to DMPC was compared with mouse wild-type apoE binding DMPC. It was found that the Arg-61 apoE bound more effectively than wild-type mouse apoE. See p. 56, ¶ 00195.

The claimed invention is directed to a gene-targeted non-human animal. The specification teaches methods to generate the claimed invention which require ES cells; however, the state of the art is such that ES cell technology is generally limited to the mouse system at present, and that only "putative" ES cells exist for other species (see Moreadith *et al.*, **J. Mol. Med.**, 1997, p. 214, S*ummary*). Note that "putative" ES cells lack a demonstration of the cell to give rise to germline tissue or the whole animal, a demonstration which is an art-recognized property of ES cells. Moreadith *et al.* supports this observation as they discuss the historical perspective of mouse ES cells as follows:

> "The stage was set-one could grow normal, diploid ES cells in culture for multiple passages without loss of the ability to contribute to normal development. Furthermore, the cells contributed to the development of gametes at a high frequency (germline competence) and the haploid genomes of these cells were transmitted to the next generation. Thus, the introduction of mutations in these cells offered the possibility of producing mice with a predetermined genotype."

Such a demonstration has not been provided by the specification or the prior or post-filing art with regard to the generation of any species of animal ES cells, other than the mouse, which can give rise to the germline tissue of a developing animal. In addition, prior to the time of filing, Mullins *et al.* (**Journal of Clinical Investigation**, 1996) report that "[A]lthough to date chimeric animals have been generated from several species including the pig, in no species other than the mouse has germline transmission of an ES cell been successfully demonstrated." (page 1558, column 2, first paragraph). As the claims are drawn to non-human animals produced by methods which require the manipulation of embryonic stem cells, and particularly since the subject matter of the specification and the claimed invention encompasses the use of such cells for the generation of a knockout animal, the state of the art supports that only *mouse ES cells* were available for use for production of the claimed null mutant mice.

This is further supported by Pera *et al.* [**Journal of Cell Science** 113: 5-10 (2000)] who present the generic criteria for pluripotent ES or EG cells [see p. 6, 2nd column] and state that, "Thus far, only mouse EG or ES cells meet these generic criteria. Primate ES cells meet the first three of the four criteria, but not the last. Numerous other candidate mammalian ES cells have been described over the years in domestic and laboratory species, but only in the mouse have all criteria been met rigorously." [See p. 6, 2nd column, last paragraph].

Accordingly, in view of the quantity of experimentation necessary for the production of gene-targeted non-human animals comprising a modified endogenous apoE4 allele, for the breadth claimed, the lack or direction or guidance, as well as absence of working examples, provided by the specification for the production of gene-targeted non-human animals comprising a modified endogenous apoE4 allele, other than the exemplified mouse, the unpredictable and undeveloped state of the art of ES cells from species other than mice, it would have required undue experimentation for one of skill in the art to make and/or use the claimed non-human animals and methods of using the same.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 1, as written, is unclear. The claim recites that the modified allele encodes a modified apoE that exhibits domain interactions "characteristic" of human apoE4. The claim is unclear because it fails to provide a clear definition of what characteristic(s) of human apoE4 the modified allele exhibits. Claims 2-4, 14 and 15 depend from claim 1.

Claim 2, as written, is unclear. The claim recites that the modification comprises a Thr → Arg substitution at a position "equivalent" to amino acid 61 of human apoE4. This is unclear because a position *equivalent* to a.a. 61 fails to

clearly define the modification that occurs on the endogenous apoE allele. Appropriate correction is requested.

Claim 14, as written, is incomplete. The claim is drawn to methods, but no clear and defined steps are recited. While minute details are not required in method claims, at least the basic steps must be recited in a positive, active fashion. See Ex Parte Erlich, 3 USPQ2d, p. 1011 (Bd. Pat. App. Int. 1986). For example, it is unclear how the determination of an effect of a test agent on a phenomenon associated with AD relates to the preamble, "A method of identifying an agent that reduces a phenomenon associated with AD...". *An effect* can result in reduction or an increase, for example. Appropriate correction is requested.

Claim 15, as written, is unclear. The claim depends upon itself. Appropriate correction is required.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the

subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-4, 14 and 15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Capecchi [**Scientific American**, 1994, 270:34-41] when taken with Dong *et al.* [**JBC**, 269:22358-22365, 1994 (cited on Applicants' IDS filed 3/5/02)] and Weisgraber [**Adv. Protein Chem.**, 45:249-302, 1994 (cited on Applicants' IDS filed 3/2/02)].

Capecchi teaches knockout technology applied to mice, specifically with respect to the disruption of the *Hox*A-3 gene and as a method of producing the same, applies to determining the *in vivo* biological function of any known gene of interest. For example, Capecchi discloses the applicability of gene targeting to many other genes, so that a correlation can be drawn between the malfunctioning gene to the manifestation of disease [see p. 41, col. 2, 2nd full paragraph]. Capecchi further discloses the essential components of a targeting vector [p. 38, col. 3, and p. 39, col. 1-2], and the steps involved for targeted gene replacement in ES cells as well as in mice [see p. 36-39 and diagrams]. Capecchi differs from the claimed invention

in that the targeting construct does not contain flanking nucleotide sequences which homologous recombine with the endogenous mouse apolipoprotein E4 to produce a Thr → Arg substitution at the position equivalent to amino acid position 61 of human apoE4

However, prior to the time the claimed invention was made, Dong *et al.* teach that human apolipoprotein E [apoE] has been implicated in the development of Alzheimer's disease. See p. 22358, 2nd col., and 1st ¶ and p. 22359, 1st col, 1st full ¶. They further teach that preferentially associates with very low density lipoproteins [VLDL] and that apoE4 associates with high density lipoproteins. They teach that site-directed mutagenesis of glutamic acid 109 in apoE3 and arginine 61 of apoE4 showed that the Arg-61 is critical in determining apoE4 liporpotein distribution, suggesting that Arg-61 interacts with the carboxyl-terminal domain to direct binding to VLDL. See *Abstract.* They teach that the interaction of either glutamic acid 109 or Arg-61 with the carboxyl terminal domain to influence interactions with lipoprotein particles was examined by mutating Glu-109 → Ala and Arg-61 → Thr. It was found that the substitution of Glu-109 → Ala did not alter the apoE3 lipoprotein distribution, whereas the Arg-61 → Thr substitution had a dramatic effect on the distribution pattern, which was converted into an apoE3 profile [i.e., the loss of Arg-61 converted apoE4 from VLDL to an HDL distribution]. As such, Arg-61 plays a key role in the determination lipoprotein preference. See Figure 8 and p. 22362, 2nd col., 1st full ¶. Dong teach that humans

are unique in having the arginine at position 61, and that in 10 species that have been examined, threonine occurs at position 61 [see p. 22364, 1st col., 1st ¶] and discuss the fact that it remains to be determined if Arg-61 also contributes to differential binding of apoE3 and apoE4 to the Aβ peptide, and that the uniqueness of the presence of the Arg-61 in humans may contribute to the pathology of Alzheimer's disease [see p. 22365, 2nd column, 2nd ¶]. Dong do not particularly teach endogenous apoE alleles from species, such as mice. However, Weisgraber teach the primary structures of apoE from 10 species, including mouse [see Figure 1, p. 250].

Accordingly, in view of the combined teachings, it would have been obvious for one of ordinary skill in the art to utilize the transgenic technology, as taught by Capecchi with a targeting vector comprising a modified apoE4 allele with a Thr → Arg substitution at the position equivalent to amino acid 61 of human apoE4 to produce transgenic mice, with a reasonable expectation of success. One of ordinary skill in the art would have been sufficiently motivated to make such a modification, as ApoE has been implicated in the pathogenesis of Alzheimer's disease, as suggested by Dong, that, "[N]o animal model appears to develop the complete pathology of Alzheimer's disease may result from the uniqueness of human apoE, specifically the presence of arginine at position 61." See p. 22364, 2nd col., 2nd ¶.

Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear and convincing evidence to the contrary.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Thái-An N. Ton whose telephone number is (703) 305-1019. The examiner can normally be reached on Monday through Friday from 8:00 to 5:00 (Eastern Standard Time), with alternating Fridays off. Should the examiner be unavailable, inquiries should be directed to Deborah Reynolds, Supervisory Primary Examiner of Art Unit 1632, at (703) 305-4051. Any administrative or procedural questions should be directed to William Phillips, Patent Analyst, at (703) 305-3482. Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center number is (703)-872-9306.

Thái-An N. Ton
Patent Examiner
Group 1632